THE EFFECT OF TEMPERATURE, FOOD, AND THE AGE OF THE CULTURE ON THE ENCYSTMENT OF *DIDINIUM NASUTUM*.

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INTRODUCTION.

Investigations on encysted organisms clearly indicate that length of life and lethal desiccation, temperature, and chemical concentration are greatly extended by encystment. Baker (1771) maintains that the rotifer, *Tylenchus*, revived after it had remained in a dried condition on the surface of grains of wheat for 27 years. Doyere (1842) found that rotifers kept for 17 days in a desiccator, followed by 28 days under the belljar of an air-pump with a pressure of only 5 to 6 cm. of mercury, and thereafter thoroughly dried in direct sunlight, and then subjected to a temperature of 140° C. were still viable. Mast says (1917): "Cysts of *Didinium* kept sealed air-tight in a 10 c.c. vial for nearly five years were still viable." Bodine found that the cysts of *Colpoda* withstand extraordinary concentrations of various acids and narcotics as well as remarkably high temperatures.

The results obtained in observations on the cause of encystment are, however, much less conclusive. Root maintains that the encystment of suctoria is due to lack of food. He says (1914): When left without food for several weeks, Podophryæ become smaller and finally encyst in situ." Mast obtained similar results in experiments on *Didinium,* but not invariably. He says (1917): "Encystment in *Didinia* can usually be induced at any time by cutting off the food supply. But it frequently occurs when there is an abundance of food present and sometimes it does not occur when there is none. This is especially true for the cultures in which conjugation has been prevented for a considerable number of generations." Miss Carter (1919) seems to think that abundant food is essential for encystment of *Amœbæ,* but that low temperature is not, although she found encysted specimens during the winter months. Miss Hogue contends that the accumulation of waste products is the primary cause of encystment. She says, referring to *Ameba limax* (1914): "The condition under which they encyst seems to point to a weakened vitality. It seems as though the digestive fluid has not been formed in sufficient quantity, owing to the rapid division. So it is not the lack of food, but rather the loss of power of assimilation." And (1917, p. 571): "When the accumulation of waste product is very great, and the *Amœbæ* have multiplied so fast that there is no further place for them to go, they encyst. I have frequently observed a culture dish containing thousands of *Amœbæ,* with plenty of food, soon become covered with cysts, the *Amœbæ* having often encysted over night."

It is generally held that encystment is a protective adaptation. Thus Calkins says (1910, p. 40): "This is a special adaptive process by which the organisms are enabled to survive when the environment is unsuitable." (P. 90) "There is a general agreement that its object is to protect the individual during periods of drought, cold, or periods of reproduction." (P. 192) "It occurs when the animal is in danger of drying, or in some cases before division, in others for the purpose of digesting a full meal." Minchin (1917) expresses similar views, as does also Jordan. Jordan says (1918, p. 73): "It serves to tide the species over a period of dryness, famine, or unsuitable temperature, and to preserve alive in a hostile environment a sufficient number of individuals until such time as favorable conditions recur. The spore-stage is in fact physiologically analogous to the periods of hibernation and estivation among the higher forms of life. In this resting state the living matter of the spore may remain dormant for years or even for decades."

It seems evident, then, that many investigators hold that encystment is induced by adverse changes in the environment, and that it protects the organism against unfavorable conditions. The evidence presented in favor of the first of these contentions is, however, not strong. A thorough experimental investigation of the relation between the environment and encystment is, therefore, highly desirable.

In this paper we shall present the results obtained in experiments on the effect of temperature, food, and the age of the food-culture on the encystment of *Didinium nasutum*.

Materials and Methods.

The didinia used in these experiments were all derived from cultures started from a stem-culture kept continuously in the Laboratory. Battery-jars were used as containers for these cultures. From time to time, as the supply of food in the culture-jars became depleted, a portion of the liquid was replaced by a corresponding quantity of a fresh, vigorous, paramecium culture. In this way the didinia were kept continuously in a flourishing and active condition.

The effect of temperature and food on encystment was ascertained as follows: Into each of five square watch-glasses, previously sterilized, was placed 3 c.c. of culture fluid containing numerous paramecia, and into each of five similar watch-glasses 3 c.c. of culture fluid taken from the same jar, but filtered so as to remove all paramecia and other organisms that might serve as food for *Didinium*. To each of the watch-glasses thus prepared there were added vigorous didinia, usually five, all taken from the same jar. Two of the watch-glasses, one with and one without food, were then placed into each of five thermostats maintained at different temperatures as indicated in Table I. All of these cultures were examined for cysts once every day until they died out. In this experiment there were consequently under observation simultaneously ten didinia cultures which were precisely the same with the exception of food and temperature. This experiment was repeated a number of times as indicated in the accompanying tables.

In a number of additional experiments there were fewer cultures and temperatures under simultaneous observation. In an extended series of experiments made after the main part of the work was completed there were only two cultures, one with and one without food, both at 27° in each experiment. Cultures containing fluid taken from the jar which had contained the didinia used in the tests were added to some of the thermostats in a few experiments. This, however, had no appreciable effect on encystment.

The results obtained in all of these experiments are summarized in Table I. These results show conclusively that at 25°–30° en-

TABLE I.

THE EFFECT OF TEMPERATURE AND FOOD ON ENCYSTMENT IN DIDINIUM.

Note that the percentage of encystment is greater in the cultures with food than in those without and that it is greatest at 25–30°, the optimum temperature for reproduction.

Temp.	With Food.			Without Food.			Ratio of Percentages of Encystment.
	No. of Cultures.	No. of Cultures Encysted.	Percentage of Cultures Encysted.	No. of Cultures.	No. of Cultures Encysted.	Percentage of Cultures Encysted.	
4–16°....	14	0	0	11	0	0	
20–23°....	26	14	53.8	22	4	18.18	3.23
25–30°....	18	15	83.3	8	3	37.5	2.22
27°.......	51	42	82.3	57	22	38.6	2.13
30–35°....	25	17	68.0	26	7	26.92	2.52
39°.......	16	6	37.5	9	1	11.1	3.38

cystment occurred in a much greater proportion of the cultures than at any other temperature, both in those with and in those without food, that at all of the temperatures excepting the lowest it occurred in a much greater proportion of the cultures which contained food than in those which did not, and that the difference in the extent of encystment in the cultures with and without food was least at 25°–30°. They show that at 39°, which is only a few degrees below the maximum, there was but little encystment, and that at 4°–16° there was none at all.

In the preceding experiments the didinia died out in relatively more cultures at the higher and the lower temperatures than at the others. At 4°–16° and at 39° they died out in over half of the cultures in two days, while at 27° they died out in less than one tenth of the cultures in the same time. Moreover, the death rate was greater at all of the temperatures in the cultures without food than in those with food. In two days at 39° two thirds of the cultures without food died out and only one half of those with food, at 30°–35° a little over one half of those without food and less than one eighth of those with food, at 27° nearly one sixth of those without food and less than one twenty-fifth of those with food, etc. Furthermore, reproduction took place more rapidly at 25°–30° than at the other temperatures. At 25°–30° the fission rate ran up in some instances to 8 a day. At 39° it never exceeded one or two a day, and at the lower temperatures of 4°–16° there was no reproduction at all.

In all of these experiments the didinia were rather suddenly subjected to the different temperatures. It was thought that the lack of encystment at the extreme temperatures might have been due to the sudden change. An extended series of tests was consequently made in which the temperature of some of the cultures was very gradually reduced to 4°–16°, and that of others very gradually raised to 39°. The results obtained in all of these tests were, however, essentially the same as those obtained in the earlier experiments.

All of the results obtained consequently indicate that encystment in *Didinium* takes place most readily under conditions of temperature and food which appear to be optimum for reproduction. The results are, however, not conclusive in reference to the question of the effect of food. An abundance of food was added in all of the experiments when they were set up and in some more was added later. In some cultures there was still an abundance of food present when encystment occurred, but in others there was none. The number of each was unfortunately not recorded. It is consequently evident that in some of the cultures which contained food encystment may have been due to absence of food. In the cultures with food there was a much greater increase in the number of didinia than in those without food. Rapid and extensive reproduction of didinia confined to a small space seems to favor encystment, and it may be that this is owing to accumulation of waste products.

In attempting to ascertain the effect of the age of the food-culture on encystment experiments were carried out as follows: Timothy hay was added to tap-water, spring-water, and distilled water in three large flasks, one gram to 100 c.c. These flasks were then kept for 30 minutes at the boiling point. After they had cooled to room temperature, usually the following day, a large number of paramecia in a small amount of liquid was added to each flask and the contents poured into battery-jars. These jars were then placed side by side and kept at room temperature. Three more cultures were prepared precisely the same way two days later and also four days later; so that at this time there were at hand three sets of paramecia cultures, one just completed, one two days old, and one four days old. A given amount of solution containing many paramecia was now taken from each of the nine cultures and put into nine square watch-glasses, each containing five vigorous didinia, all taken from the same jar. These watch-glasses were put into a thermostat kept at 27°–28°. The number of cultures in which cysts occurred was recorded daily, as well as the number of cysts in each and the condition of the paramecia.

Other sets of watch-glass cultures were prepared and treated precisely like this on the following days. A summary of the results obtained in all of these are presented in Table II.

Table II.

The Relation between the Age of the Food-culture and Encystment in Didinium.

Age of Food Culture in Days.	No. of Didinia Cultures.	No. of Cultures Encysted.	Average Time Required for Encystment in Days.	Percentage of Cultures Encysted.
0	11	2	3	18.1
1	8	5	3-2/5	62.5
2	11	4	3	36.3
3	14	7	3	50
4	14	10	3-1/5	71.4
5	20	15	3-1/5	75
6	15	14	3-1/14	93.3
7	21	11	2-6/11	52.3
8	18	12	3-1/3	66.6
9	13	9	2-2/9	69.2
10	15	7	3	46.6
11	10	4	3	40
12	10	4	2-3/4	40

By referring to this table it will be seen that as the culture medium increased in age the percentage of the number of cultures in which encystment occurred increased to a maximum, after which it decreased. It is well known that the chemical composition of protozoa cultures changes with age. It is consequently evident that the increase and decrease in the percentage of encystment noted must have been due either to this change or to a change in the quantity or the quality of the food. The amount of food was, however, practically the same in all of the didinia cultures, but the quality may have been different. It is therefore impossible to say whether the change in the percentage of encystment was due to a change in the chemical composition of the culture medium or to a change in the quality of the food. However this may be, encystment occurs freely in the culture media which are very favorable for growth and reproduction of paramecia. The maximum was found in cultures six days old, when the paramecia were very abundant and vigorous. Whether or not fission rate was at a maximum at this time was, however, not ascertained.

Summary.

1. Didinia encyst most readily at a temperature of 25°–30°, which is also the optimum temperature for growth and fission.

2. They do not encyst in temperature so low or so high that it is injurious. They do not encyst at all below 16° and rarely above 39°.

3. They encyst more freely in cultures supplied with food than in those without food, but this is probably due to greater increase in numbers, resulting in greater accumulation of waste material in the one than in the other.

4. They encyst most readily in culture media, which are probably most favorable for growth and reproduction of the paramecia on which they feed.

5. Encystment serves as protection against unfavorable conditions in reference to food and temperature, but such conditions do not facilitate encystment.

6. Encystment is probably induced by the accumulation of excretory waste material.

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